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Effects of *Pediococcus*- and *Saccharomyces*-based probiotic (MitoMax®) on coccidiosis in broiler chickens

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Abstract

Coccidiosis is the major parasitic disease of poultry. In this study, the role of the commercial probiotic MitoMax® which contains *Pediococcus acidilactici* and *Saccharomyces boulardii* was evaluated by measuring body weight gain, fecal oocyst shedding, and serum antibody responses as an alternative control method of prophylactic drug against coccidiosis. Day-old broiler chicks were fed regular or probiotic diets supplemented with MitoMax at 0.01%, 0.1%, or 1.0% of diet, and challenged 2 weeks later with 5000 oocysts of either *Eimeria acervulina* (EA) or *Eimeria tenella* (ET). Birds fed 1.0% or 0.1% MitoMax-supplemented diets in EA- or ET-infected groups shed less ($P<0.05$) oocysts than control-infected chickens. Also, chickens fed 0.1% MitoMax-supplemented diet and infected with EA exhibited higher ($P<0.001$) serum *Eimeria*-specific antibodies than other groups. These results demonstrate that MitoMax

Abbreviations: REG, Regular diet; M 0.01, 0.01% of MitoMax®; M 0.1, 0.1% of MitoMax®; M 1.0, 1.0% of MitoMax®; DPI, Days post-inoculation; OD, Optical density

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may enhance the resistance of birds against coccidiosis by enhancing humoral immunity when included at $\geqslant 0.1\%$ of the broiler diet.

Keywords: Probiotic; MitoMax; Broiler; *Eimeria*; Coccidiosis; Humoral immunity

Résumé

La coccidiose est une parasitose aviaire majeure. Comme mesure alternative de prophylaxie médicale pour cette affection, nous avons étudié le rôle du probiotique commercial MitoMax, qui contient *P. acidilactici* et *S. boulardii*, en mesurant le gain de poids, l'excrétion d'oocystes, et les réponses anticorps. Des poulets de chair d'un jour ont reçu des régimes normaux ou probiotiques additionnés de 0.01%, 0.1% ou 1.0 de MitoMax, et ont été éprouvés deux semaines plus tard avec 5000 oocystes soit de *Eimeria acervulina* (EA), soit de *Eimeria tenella* (ET). Les oiseaux des groupes infectés avec EA ou ET ayant reçu les régimes supplémentés avec 1.0% ou 0.1% de MitoMax ont excrété moins d'oocystes ($P<0.05$) que les animaux des groupes infectés contrôles. De plus, les animaux nourris avec le régime supplémenté avec 0.1% de MitoMax et infectés avec EA ont développé plus d'anticorps spécifiques d'Eimeria ($P<0.001$) que les animaux des autres groupes. Ces résultats montrent que le MitoMax est susceptible d'améliorer la résistance des volailles à la coccidiose en augmentant l'immunité humorale, par incorporation à environ 0.1% dans la ration alimentaire des poulets d'élevage.

Mots clés: Probiotique; MitoMax; Poulets; *Eimeria*; coccidiose; l'immunité humorale

1. Introduction

Avian coccidiosis is one of the most economically important diseases of the poultry industry [1]. As the world's poultry industry continues to expand, so does the concern for the control of coccidiosis, where increasing drug-resistance and drug-residue problems in food products threaten to ban chemotherapeutic control methods [2]. To prevent the emergence of drug-resistant strains of *Eimeria*, the etiologic agent of coccidiosis, different types of drugs are administered on a rotational basis and/or in combination with existing live vaccines [3,4]. Broad-spectrum immunological prophylaxis is a desirable alternative for control of coccidial infections in the poultry industry.

Recent evidences [5–8] that various dietary and microbial supplements can influence host immunity against various diseases prompted us to investigate the role of a new probiotic (MitoMax®, Imagilin Technology, LLC, Frederick, MD) on avian coccidiosis. This probiotic consists of live *Pediococcus acidilactici* and *Saccharomyces boulardii*. Pediococci exert antagonism against other microorganisms primarily through the production of lactic acid, in addition to the production of antimicrobial peptides known as pediocins by some strains [9]. *S. boulardii* is an effective and safe treatment for prevention of antibiotic-associated diarrhea when given concomitantly to patients receiving *Helicobacter pylori* eradication [10]. We

hypothesized that these live microbial supplements contained in MitoMax may compete and interfere with *Eimeria* invasion, thus enhancing the bird's resistance and minimizing the negative effects associated with coccidiosis including clinical symptoms such as diarrhea. The purpose of this study was to evaluate the efficacy of the probiotic MitoMax as a natural alternative to chemotherapeutic drugs for the prevention of coccidiosis.

2. Materials and methods

2.1. Birds and treatments

Ninety day-old broiler chicks were randomly assigned to nine cages ($N = 10$/cage) of an electrically heated battery; 30 birds fed a corn-soybean-based regular broiler diet serving as a control (REG), while 60 birds were assigned to three goups (20 birds/group) receiving similar diets with the probiotic MitoMax added at the rate of 0.1 g/kg feed (M 0.01), 1 g/kg feed (M 0.1), or 10 g/kg feed (M 1.0). The diets were formulated to meet the nutrient requirements for broilers as recommended by the National Research Council [11]. All birds were maintained on the same treatment diets provided ad libitum and body weights were recorded 0 and 10 days post-inoculation (dpi) with *Eimeria* oocysts. All experiments were performed according to the guidelines established by the Beltsville Area Institutional Animal Care and Use Committee (IACUC).

2.2. Eimeria infection and assessment of fecal oocyst production

Forty 14-day-old birds (10 birds/treatment) were selected on mean weight-basis within each treatment, wing-tagged, placed at 2 birds/cage, and inoculated esophageally with 5000 *E. acervulina* (EA) or *E. tenella* (ET) sporulated oocysts. Oocyst production and shedding were assessed as described [12]. Briefly, fecal droppings from each cage (3 cages/treatment) were collected for 5 d, starting on the 6th dpi, fecal material ground and homogenized, and two 35 ml samples were taken and diluted, and the oocysts were counted microscopically using a McMaster counting chamber. The total number of oocysts was calculated using the formula: total oocysts/bird = oocyst count × dilution factor × (fecal sample volume/counting chamber volume)/number of birds per cage.

2.3. ELISA for determination of serum antibody levels

Blood samples were obtained 10 dpi from individual birds ($N = 3$/group), allowed to clot at 4 °C for 4 h, and the sera collected. Serum samples were tested for antibodies against *Eimeria* using ELISA as described [13]. Briefly, microtiter plates were coated overnight with 10 μg/well of the recombinant coccidial antigens 3-1E [14] or EtMIC2 [15], washed with PBS-0.05% Tween, and blocked with PBS-1% BSA. Serum dilutions (1:50; 100 μl/well) were added, incubated with

continuous gentle shaking, washed, and bound Ab detected with peroxidase-conjugated rabbit anti-chicken IgG (Sigma) and peroxidase-specific substrate. Optical density (OD) was determined at 450 nm with a microplate reader (Bio-Rad, Richmond, CA).

2.4. Statistical analysis

All values are expressed as mean ± S.D. Mean values for body weight gains and antibody response were compared between control (uninfected REG) and *Eimeria*-infected groups by the Dunnett multiple comparisons test and mean values for fecal oocyst shedding were compared among EA- or ET-infected groups by the Duncan's Multiple Range test following ANOVA using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). Differences between means were considered significant at $P < 0.05$.

3. Results

3.1. Body weight gains

There was no significant difference in body weight gains among the groups fed regular or MitoMax-supplemented diets and among the groups uninfected or infected with EA or ET (Fig. 1). However, body weight gains were 3.4% and 10% lower in the groups infected with EA and ET, respectively, compared with those of uninfected control birds fed regular diets (REG). In EA-infected groups, weight gains of birds on the M 0.01 and M 1.0 diets were 6.3% and 2.9%, respectively, higher than those of birds fed REG diet. In ET-infected groups, weight gains of birds on the M 0.1 and M 1.0 diets were 14.5% and 6.7%, respectively, higher than those of birds fed REG diet.

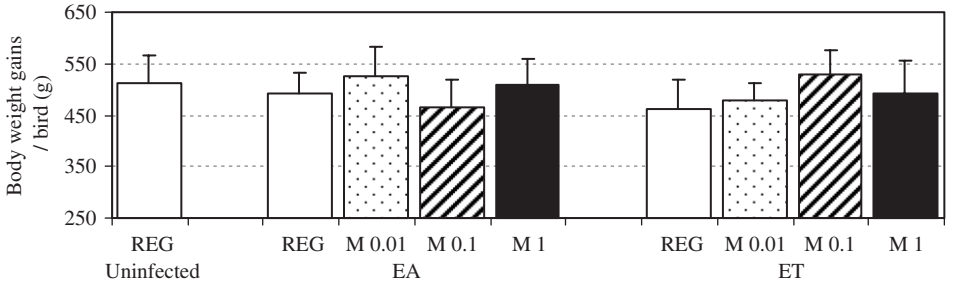

Fig. 1. Body weight gain for 10 d after inoculation with *E. acervulina* (EA) or *E. tenella* (ET) from hatching of broiler chickens fed regular (REG), 0.01% (M 0.01), 0.1% (M 0.1), or 1.0% (M 1.0) MitoMax-supplemented diets. Birds were infected with 5000 oocysts of either EA or ET at day 14 post-hatch. Each bar represents the mean ± S.D. ($N = 10$).

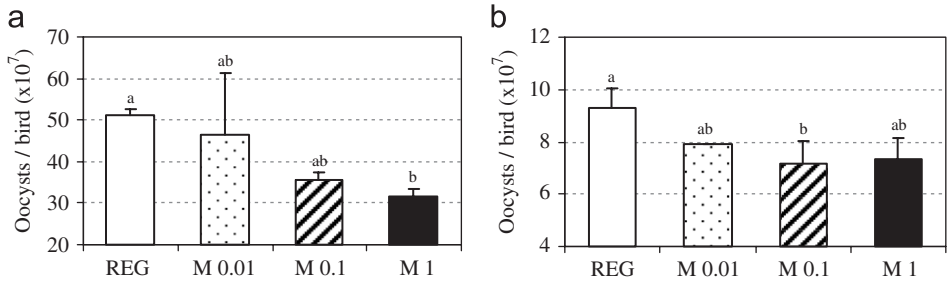

Fig. 2. Fecal oocysts shed by birds infected with *E. acervulina* (a) or *E. tenella* (b). Each bird was inoculated with 5000 EA or ET oocysts at day 14 post-hatch. Each bar represents the mean ± S.D. ($N = 3$). Values with the different alphabet character for the same factor are significantly different at $P < 0.05$, as assessed by Duncan's multiple range test.

3.2. Oocyst shedding

In birds infected with EA, REG, M 0.01, M 0.1, and M 1.0 groups shed 51.2×10^7, 46.4×10^7, 35.4×10^7, and 31.6×10^7 oocysts/bird, respectively (Fig. 2a). Probiotic-fed chickens showed 10–38% reduction in the number of oocysts shed after infection with EA, and the significant ($P < 0.05$) difference from REG was found in M 1.0 group. Further, ET-infected chickens shed 9.3×10^7, 7.9×10^7, 7.1×10^7, and 7.3×10^7 oocysts/bird, respectively, in REG, M 0.01, M 0.1, and M 1.0 groups (Fig. 2b), and birds of the M 0.1 group shed significantly ($P < 0.05$) fewer oocysts.

3.3. Serum antibody responses

Eimeria-specific antibody responses to 3-1E and EtMIC2 antigens in infected birds fed regular or probiotic diets are shown in Fig. 3. Antibody responses against 3-1E or EtMIC2 were higher in the groups infected with EA or ET compared to those of uninfected control group. Significant ($P < 0.001$) elevation of antibody responses to 3-1E was found in birds fed 0.1% MitoMax-supplemented diet (M 0.1) and infected with EA or ET 10 dpi (Fig. 3a). Also, in the same birds infected with EA, antibody responses to EtMIC2 was significantly ($P < 0.001$) higher than those of uninfected control birds (Fig. 3b).

4. Discussion

Coccidiosis caused by *Eimeria* is an important disease in intensive poultry production, leading to reduced growth and sometimes death in broiler chickens with significant economic losses of up to $3 billon annually worldwide [16,17]. The present work investigated the effects of a new probiotic (MitoMax) on susceptibility of chickens to coccidiosis. Weight gain is usually used as a parameter of infection following *Eimeria* challenge [18,19]. During *E. tenella* infection, chickens fed 0.1% MitoMax apparently gained more weight than birds of other groups although such

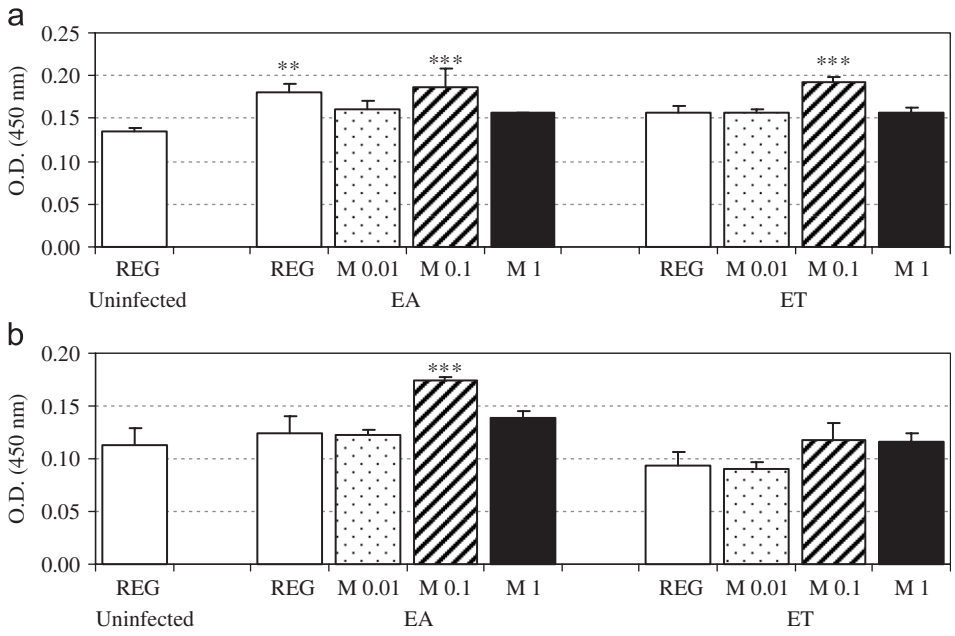

Fig. 3. Antibody responses against 3-1 E (a) and EtMIC2 (b) in broiler chickens fed regular, 0.01%, 0.1%, or 1.0% MitoMax-supplemented diets and infected with *E. acervulina* or *E. tenella*. Birds were infected with 5000 EA or ET oocysts at day 14 post-hatch. Each bar represents the mean ± S.D. ($N = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$.

difference was not significant. One of the reasons why MitoMax had no significant effect on the birds' weight gain may be attributed to the fact that this probiotic was initially developed for pets with controlling body weight in mind. Similar probiotic effect on weight gain in mice has been reported when using *S. boulardii* [18], but more direct influence of *S. boulardii* has been shown on feed efficiency in broilers infected with *Salmonella enteritidis* [19]. Therefore, comparative effects of *Pediococcus* and *Saccharomyces* on body weights in birds treated for various lengths of time and also infected with different doses of *Eimeria* need to be further investigated. In regards to oocyst shedding, all groups fed MitoMax diets and infected with either *E. acervulina* or *E. tenella* shed less oocysts than non-probiotic groups. There was negative correlation between oocyst shedding and body weight gains in this study and similar correlation between weight gain and the numbers of excreted oocysts has been previously reported in other studies on coccidiosis [8,15]. Although it is desirable to see positive effects with both parameters, direct correlation between increased weight gain and reduced oocyst shedding has not always been the case with probiotic research [7,12,20]. This aspect should then be considered in future studies with MitoMax where various infection doses would be implemented to provide more reliable information on protective immunity to *Eimeria*.

Examining humoral immune responses best demonstrated the positive effects of the probiotic. In humoral immunity study, the group fed the probiotic MitoMax at

0.1% of the diet (M 0.1) exhibited the highest antibody levels following EA or ET infection whereas no significant humoral response was present in M 0.01 and M 1.0 birds compared to those of uninfected control. A number of studies have demonstrated that natural foods or ideal vaccines may protect against *E. acervulina* or *E. tenella* by inducing higher levels of cell-mediated immunity [6,13,20]. Probiotics containing lactic acid producing bacteria enhance immune responses and defense activities against undesirable microorganisms and such protection has been partially attributed to increased innate immune response [7,20,21].

Overall, we investigated for the first time the effects of a *Pediococcus*- and *Saccharomyces*-based probiotic (MitoMax) on susceptibility of chickens to coccidiosis in a quest for new ways to control the disease. MitoMax showed immune-enhancing activity as reflected by reduced oocyst shedding at 0.1% or 1.0% of diets and better antibody response against *Eimeria* at 0.1% of diet in chickens fed MitoMax although body weight gains were not statistically improved. The enhancement of humoral immunity carry much promise for the use of MitoMax to boost the resistance of birds and protect against coccidiosis. These results demonstrate that MitoMax may enhance the resistance of birds against coccidiosis and enhance humoral immunity when included at $\geqslant$0.1% of the broiler diet. As the list of potential alternative control agents grows, more research is needed to better characterize the mode of actions of these agents in order to optimize their use as alternative control methods and safe immunomodulators in poultry.

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